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Minireview

Transcriptional regulation of metastatic [Id]entity by KLF17

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Abstract

A novel *in vivo* screening approach has identified *KLF17* as a key metastasis suppressor gene that acts through regulation of Id1 transcription factor-dependent induction of the epithelial-to-mesenchymal transition.

The development and growth of a malignant tumor relies on molecular changes that affect the function of cellular proto-oncogenes and tumor suppressors [1]. Certain alterations are required constitutively during tumor initiation and progression, whereas others are more specifically associated with aspects of tumor metastasis - the spread of malignant cells to secondary tissues. Metastasis suppressors are proteins that specifically repress events associated with metastasis without affecting primary tumor growth [2]. In a recent paper in *Nature Cell Biology*, Huang and colleagues (Gumireddy *et al.* [3]) describe experiments that identify a new metastasis suppressor, KLF17.

The course of metastasis

Progression towards metastatic disease involves a distinct sequence of events. Tumor cells attract endothelial cells to promote angiogenesis; dissociate from the primary tumor mass and migrate towards endothelial cells; intravasate through endothelial cells and the surrounding matrix; enter the intra-tumoral vasculature; exit the vasculature at the secondary sites; infiltrate the new environment; and establish growing malignant colonies within a new organ [4]. These events are likely to be driven in large part by changes in transcriptional programs that affect expression of genes required for these processes (for example, genes that promote angiogenesis, cell adhesion and matrix proteolysis). These transcriptional changes are mediated by alterations in the tumor microenvironment [5] as well as by genetic alterations in proto-oncogenes and tumor suppressors [6]. For example, many reports have shown that oncogenic mutations in *H-Ras* or loss of p53 tumor suppressor function induce transcriptional programs that promote metastasis through alterations in the expression of genes required for the invasion of cancer cells into the surrounding tissues [4,7-10]. Transcription factors that promote the loss of epithelial characteristics, such as (cell-to-cell) adhesion, have also been proposed to play a key role in the initiation of metastasis.

The epithelial-to-mesenchymal transition (EMT) was initially described as a fundamental process that drives morphogenetic tissue movements in animal embryos [11]. Increasing evidence supports the hypothesis that epithelial tumors adopt EMT-like characteristics during the invasion of proximal tissues [12,13]. EMT involves the loss of apico-basal epithelial cell polarity and the development of a migratory polarity (leading edge and trailing edge) that promotes migration [14]. The molecular hallmarks of EMT include the increased expression of transcription factors that contribute to the loss of cell adhesion through the downregulation of the cell adhesion molecule E-cadherin and increased proteolysis of the surrounding extracellular matrix as a result of the elevated expression of matrix metalloproteinases [14]. Cells that have undergone EMT in culture show an increased capability for invasive behavior *in vitro* and *in vivo* [12].

Genome-wide screen for metastasis suppressors

Gumireddy *et al.* [3] used a genome-wide screen to identify genes that induce metastasis when their expression is attenuated. Non-invasive mouse mammary tumor cells were transduced with a genome-wide short hairpin RNA (shRNA) library and implanted into mouse mammary fat pads. Lung metastases that developed in these animals were then analyzed by PCR for the most abundant shRNA transcripts, one of which was found to target expression of Krüppel-like transcriptional factor 17 (KLF17). Gumireddy *et al.* [3] then found that shRNA-mediated attenuation of KLF17 expression in the MCF-7 line of human non-metastatic breast cancer cells promoted lung metastases in mice into which the treated cells were introduced. In addition, the overexpression of KLF17 in highly metastatic 4T1 mouse breast cancer epithelial cells decreased the number of lung metastases in mice compared to control cells. These experiments provide strong evidence that *KLF17* is a new metastasis suppressor gene.

KLF17 belongs to the Sp (small protein)/KLF zinc-finger protein family. Other members of this family have been shown to regulate cell invasion *in vitro* [15]. For example, ectopic expression of KLF4 and/or KLF5 blocked the invasive behavior of esophageal cancer cells *in vitro* [15].

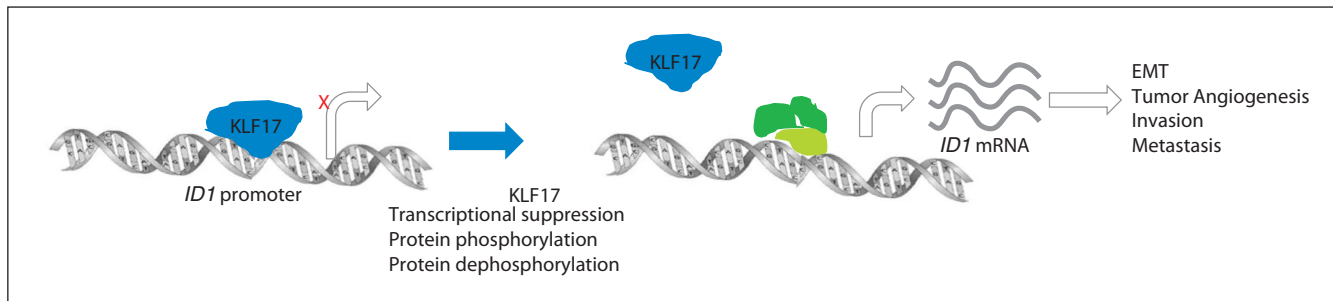


Figure 1

Regulation of *ID1* expression by KLF17. *ID1* transcription is suppressed by KLF17 binding to the *ID1* promoter. Loss of expression of KLF17 by transcriptional suppression or inactivation by post-translational modification (possibly by phosphorylation or dephosphorylation) leads to transcription of *ID1* through positive transcriptional regulators (shown in green) and induction of the multiple processes regulated by its protein product, Id1.

However, the exact mechanisms by which these proteins achieve their suppressive effect was not understood. Gumireddy *et al.* [3] employed microarray analysis of mRNAs in cells subjected to loss or gain of *KLF17* expression to provide insights into the mechanisms by which KLF family members suppress cell invasiveness and metastasis. They show in particular that shRNA-mediated attenuation of *KLF17* levels in non-metastatic cells induces an EMT 'signature' of gene expression and increased expression of inhibitor of differentiation-1 (*Id1*), which is known to be a negative regulator of basic helix-loop-helix family transcription factors and to be involved in promoting EMT. Conversely, overexpression of *KLF17* suppressed *Id1* expression. The authors further show that *KLF17* suppresses *Id1* expression directly by binding to its promoter region. *Id1* and *KLF17* have opposite effects on a tumor's ability to metastasize. Primary tumor samples with high *Id1* and low *KLF17* expression were highly metastatic, whereas those with higher expression of *KLF17* and lower levels of *Id1* were less metastatic. Taken together, these data support the conclusion that *Id1* plays a critical role in the regulation of metastasis by *KLF17*.

Id1 and human cancer

ID1 is overexpressed in various human cancers, including endometrial, ovarian, prostate and breast cancers [16], and had previously been shown to promote invasion and metastasis [10]. In tubular epithelial cells, *ID1* was shown to be required for transforming growth factor- β -mediated loss of E-cadherin expression (a molecular hallmark of EMT) [17]. *ID1*-mediated downregulation of E-cadherin occurs through the direct binding of *Id1* protein to the transcription factor HEB (HELA E-box binding factor), which prevents HEB from accessing the E-cadherin promoter [17]. Interestingly, Gumireddy *et al.* [3] show that the loss of *KLF17* function in the NMuMg mouse mammary epithelial cell line transformed by the oncogene *V12H-Ras* enhances its capacity to produce lung metastases and drives metastasis to

several other organs. Given that *V12H-Ras* induces EMT [18-20], these results suggest that processes in addition to EMT contribute to the promotion of metastasis by *Id1*. For example, *Id1*-induced metastasis could also involve tumor vascularization, as *Id1* promotes production of vascular endothelial growth factor (VEGF) in prostate cancer cells [21]. Taken together, these studies suggest a model in which loss of *KLF17* leads to induction of *ID1*, which could promote primary tumor vascularization via VEGF production and initiate invasion through EMT-associated processes, including loss of E-cadherin adhesions and increased activation of matrix metalloproteinases.

One important question raised by the work of Gumireddy *et al.* [3] is whether the regulation of *KLF17* is entirely transcriptional or if its activity is also regulated post-translationally, and what upstream factors control gene expression or protein activity. The transcriptional activity of Sp1/KLF family members has been shown to be regulated post-translationally by phosphorylation [8,22]. A search for phosphorylation-site motifs using Scansite [23] predicts a high-stringency target site for phosphorylation by protein kinase C- ϵ (PKC ϵ) within the amino-terminal region of *KLF17*. Given that the activity of some Sp1/KLF family members is known to be regulated by PKC isoforms [8,24-26], it is plausible that the ability of *KLF17* to suppress *ID1* transcription could be either positively or negatively tuned by some PKCs (Figure 1). However, future experiments addressing the mechanisms of *KLF17* regulation will be needed to fill this gap in our understanding of metastatic progression.

The findings of Gumireddy *et al.* [3] highlight the feasibility and the value of *in vivo* loss-of-function screens. As the authors point out, these screens should be carried out in models other than breast cancer, as distinct transcription factors might play a role in the regulation of invasion and metastasis in different cancer types.

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